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EPIDEMIOLOGICAL AND EPIZOOTIOLOGICAL INVESTIGATIONS OF FILOVIRUSES IN THE CENTRAL AFRICAN REPUBLIC

ANNUAL REPORT

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A member of the filovirus group is active in the CAR, as demonstrated by significant seropositivity rates in human populations as well as in different animal species (rodents, dogs, cattle, chicken). Infection by this virus seems not usually associated with severe illness or hemorrhagic signs.
Further studies are needed : 1) isolate and characterize the member of filovirus group, 2) establish the biological significance of filovirus antibodies, 3) define the epidemiology of filoviruses in CAR.

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S U M M A R Y

The collaborative program of sero-epidemiological investigations on Central African Hemorrhagic Fever Viruses (specially Filoviruses), has been focused in 1987 and 1988 on two cooperative villages, Gordil and Ouandja, of the Vakaga district (Northen part of the country), which have been studied since 1985.

There are serological evidences of the active circulation of an antigenically related member of the Filovirus group. The infection can occur early in the life and inter-human contamination is not to be excluded.

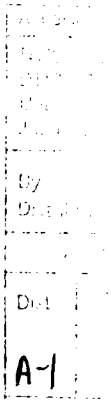
As very few cases of severe illness seems to be associated with infection by this virus in the CAR, and as the antigenic specificity of the serological test used is not known, one cannot assert if the virus responsible for the high rate seropositivity is Ebola, Marburg or an antigenically related strain.

For similar reasons, despite the obtention of numerous serological and virological data, the role of animals (rodents, cattle, chicken, goats, sheeps, dogs) in the natural cycle of filoviruses cannot be still clearly established.

Further studies are needed : to isolate this virus, to determine whether the antibodies detected are specific of Ebola or Marburg virus, or can neutralize these viruses, and to study the Filovirus epidemiology in the CAR by establishing a meticulous survey of seropositive and seronegative families.

FOREWORD

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.



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BODY OF THE REPORT

A. STATEMENT OF THE PROBLEM

In the past, the filoviruses, a new group of pathogenic agents, were not adequately or thoroughly studied in Africa. The current Institute Pasteur/USAMRIID filovirus field program was established to systematically study these agents and accurately assess and reduce their natural threat by defining their epidemiology, ecology, pathogenicity, and by developing procedures to control disease.

The current field program, as outlined in previous reports (1,2), was divided into three phases. Phase one consisted of cross- sectional serosurveys conducted in different ecological or phytogeographic areas to locate areas of virus activity as measured by the prevalence of filovirus reactive antibody. Phase two consisted of prospective studies conducted in well defined populations to document active virus transmission by identifying seroconversions. Phase three consisted of in depth studies to identify the occurence of clinical diseases associated with antibody prevalence and define the local ecology of filovirus activity. Phase one was completed and phase two started in 1986. The results of the phase one studies on the epidemiology of filoviruses in CAR (Central African Republic) suggested that the research efforts should be formed on the Vakaga district in the northern part of the country : 1. The filovirus activity as measured by antibody prevalence was highest in this region; 2. Virus activity was confined to select villages; 3. The antibody prevalence was highest among the female population; 4. Both Ebola virus and Marburg virus were active in the Vakaga district; and 5. The numerous double seropositives (Ebola virus and Marburg virus reactive antibody positives) suggested shared risk factors for the filoviruses.

Unfortunately, our follow-up study revealed that conducting long term prospective studies in the Vakaga would be difficult. Many villages, Amardjedi and Sikikede for example, were not willing to participate in additional studies. The reluctance of Amardjedi and Sikikede residents to participate in long term studies was due to their support of illegal poaching and their fear that outsiders would notify the authorities of their activities.

In light of the reluctance of many villages to cooperate and the logistical problems encountered in traveling deep into the Vakaga district, two cooperative villages, Gordil and Ouandja, were selected for detailed studies in 1987 and 1988. The results of cross sectional studies revealed that the antibody prevalence in villages that neighbored Gordil and Ouandja did not significantly differ from the antibody prevalence in Gordil and Ouandja, indicating that the results generated from studies in these two villages would reflect the epidemiology and ecology of the region.

Unfortunately we encountered also in 1988 the reluctance of inhabitants of Gordil, as they told us they had been promised by previous mission staff to be provided with wells and other goods. Of course it was impossible to dig wells considering our capacities.

Taking account of the results of the 1987 serosurvey (3), we focused in 1988 our studies on select populations : children less than 6 years old, families with seroconversion cases or with numerous seropositives, dogs, rodents caught in fields owned by seropositive and seronegative families.

B. METHODS

1. Sampling procedures:

In brief, the following sampling procedure was implemented. In each village, volunteers were selected, questionnaires consisting of general background questions were completed for each individual, blood samples were drawn, and each participant was given an identification number, medical examination, and treated for illnesses. Children less than 6 years old included in the 1988 survey were vaccinated against Yellow Fever. In each village a preliminary census has been conducted, maps drawn and heads of all households identified.

Blood samples were also taken on different animal species, especially animals living in contact with man : rodents, dogs, chickens, cattle.

2. Serological procedures:

Blood samples were drawn and allowed to clot and the resulting serum specimen was divided into two aliquots. One aliquot was screened at the Institute Pasteur and one aliquot was tested at USAMRIID. The serum samples were thawed and separated into three aliquots. Two aliquots were stored frozen at -20°C, while the remaining aliquot was diluted and screened double blind by IFA for hemorrhagic fever virus reacting antibodies. The samples were screened at a 1:16 dilution on CRELM slides and the seropositives were screened and titrated on hemorrhagic fever virus infected and uninfected Vero cell monospecific spot slides.

Specimens were considered positive if they reacted with virus infected and not virus uninfected Vero cell monospecific spot slides. Specimens were considered positive if they reacted with virus infected and not virus uninfected Vero cells. The endpoints for the serological titrations were recorded as the reciprocal of the last dilution which produced a positive react with infected cells. The serological results cited in reports are those obtained at USAMRIID Laboratory, but one also will found the Institute Pasteur's own results obtained in 1985,1986, 1988.

In 1988, not only the same sera found to be positive by USAMRIID laboratory were positive too in our hands, but also some others which poses the problem of the specificity of the only IFA test for assessing Filovirus activity.

C. RESULTS

1. Filovirus antibody prevalence :

The prevalence seems lower in 1988 than the one observed during the period 1985-1987 (Tables I, II). In Ouandja this antibody decreasing seems to be related to an unusual weak prevalence in females, especially less than 30 years old (Table III), whereas the 1988 prevalence in males is not significantly different from the 1985-1987 prevalence.

Despite this new feature the overall prevalence in Gordil and Ouandja for the period 1985-1988 remains high and similar (22.8% in Gordil, 24.9% in Ouandja). None of the human sera collected in 1988 reacted with Marburg antigen.

An encampment of Mbororo people (nomads travelling with their cattle herd) was also investigated, the overall Filovirus antibody prevalence was 4.5% (1 positive/22 tested).

2. Seroconversions, seroreversions :

Unlike the previous serosurveys (3), no seroconversion has been observed in 1988. On the contrary several seroreversions occured between 1987 and 1988 (Table IV). Especially, some high titered seropositive persons in 1987 were found seronegative in 1988, and 3 persons who seroconverted between 1986 and 1987, then seroreversed between 1987 and 1988.

3. Seroprevalence in select animal populations :

Whereas antibody prevalence in dogs was high in 1987 (3), no dog serum has been found positive in 1988 (Table V). On the other hand the prevalence in rodents is comparable, indicating a low infection level in these mammals caught near houses or in croplands as well

Table I : Filovirus antibody prevalence in 1985, 1986, 1987 and 1988, previously unsurveyed Gordil populations.

Survey Year	LAB Identif*	General Total	General N° Pos	General % Pos	Male Total	Male N° Pos	Male % Pos	Female Total	Female N° Pos	Female % Pos
85	USAMRIID-IPB	147	30	20.4	92	19	20.6	55	11	20.0
86	USAMRIID-IPB	62	10	16.1	27	3	11.1	35	7	20.0
87	USAMRRID	89	34	38.2	33	12	36.4	55	20	36.6
88	USAMRIID	30	0	0.0	17	0	0.0	13	0	0.0
88	I.P. BANGUI	30	3	10.0	17	1	6.0	13	2	15.0

Only those individuals not surveyed in previous years are shown in this table.

*Workers for I.P. Bangui : 1985 Alain J. GEORGES and Jean Paul GONZALEZ, 1986 J.P. GONZALEZ and Didier MEUNIER, 1987 no aliquot available for IPB, hence not tested in Bangui according to joined agreement IPB-WRAIR 1988: Christian MATHIOT, Alain J.GEORGES.

(a) Same samples than previous data shown by USAMRIID 1988, Not considered for establishing percentages (discrepency).

Table II : Filovirus reactive antibody prevalence in 1985, 1986, 1987 and 1988 previously unsurveyed Ouandja populations.

Survey Year	LAB Identif*	General Total	General N° Pos	General % Pos	Male Total	Male N° Pos	Male % Pos	Female Total	Female N° Pos	Female % Pos
85	USAMRIID-IPB	135	40	29.6	64	12	18.8	71	28	39.4
86	USAMRIID-IPB	105	14	13.3	47	4	8.5	58	11	18.9
87	USAMRIID	219	73	33.3	103	24	23.5	116	49	40.5
88	USAMRIID	79	7	8.8	38	5	13.2	38	2	5.3
88	I.P.BANGUI(a)	76	13	16.0	38	7	18.0	38	6	16.0

Only those individuals not surveyed in previous years are shown in this table.

*Workers for I.P. Bangui : 1985 Alain J. GEORGES and Jean Paul GONZALEZ, 1986 J.P. GONZALEZ and Didier MEUNIER, 1987 no aliquot available for IPB, hence not tested in Bangui according to joined agreement IPB-WRAIR 1988: Christian MATHIOT, Alain J.GEORGES.

(a) Same samples than previous data shown by USAMRIID 1988, Not considered for establishing percentages (discrepency).

Table III : Age and sex distribution of filovirus antibodies in previously unsurveyed populations (1988). (C.MATHIOT, P.M.V.MARTIN, M.C.GEORGES-COURBOT).

Age	Females			Males			General		
	Total	N° Pos	% Pos	Total	N° Pos	% Pos	Total	N° Pos	% Pos
01-06	20	0	0.0	24	3	12.5	44	3	6.8
06-10	4	0	0.0	7	1	14.3	11	1	9.1
11-20	5	0	0.0	2	0	0.0	7	0	0.0
21-30	2	0	0.0	0	0	0.0	2	0	0.0
> 30	7	2	28.6	5	1	20.0	12	3	25.0

Table IV : Filovirus seroreversions in Ouandja and Gordil between 1986 and 1988.(C.C.MATHIOT, P.M.V.MARTIN, M.C.GEORGES-COURBOT)

House	sex / age	1986			1987			1988		
		Es	Ez	Mbg	Es	Ez	Mbg	Es	Ez	Mbg
Ouandja:										
12	M/7	0*	0	0	32	256	0	0	0	0
13	M/11	0	0	0	128	128	0	0	0	0
14	M/65	16	16	0	64	128	0	0	0	0
24	M/16	NT	NT	NT	32	128	0	0	0	0
39	M/10	NT	NT	NT	1024	1024	0	0	0	0
	M/8	NT	NT	NT	128	256	0	0	0	0
46	F/35	NT	NT	NT	128	512	0	0	0	0
48	F/41	64	128	0	256	512	0	0	0	0
54	F/41	16	32	0	128	128	0	0	0	0
72	M/15	0	0	0	128	512	0	0	0	0
	F/9	NT	NT	NT	64	0	0	0	0	0
84	F/33	32	64	0	256	256	0	0	0	0
	M/12	NT	NT	NT	32	128	0	0	0	0
	M/6	NT	NT	NT	128	64	0	0	0	0
Gordil:										
B16	F/9	NT	NT	NT	128	32	0	0	0	0

* Titres against Ebola Sudan (Es), Ebola Zaire (Ez), Marburg (Mbg)
NT not tested

Table V : Prevalence of Filovirus reactive antibody prevalence in animal serosurveys 1987 and 1988

SURVEY SPECIES	YEARS SURVEY	TOTAL N°	FILOVIRUSES POS		CCHFV POS		RVFV POS		LASSA POS	
			N°	%	N°	%	N°	%	N°	%
Cattle	87	20	2	10	4	20	0	0	0	0
	88	26	4	15.4	3	11.5	0	0	0	0
	87	131	5	3.8	0	0	0	0	4	3.1
Chickens	88	50	1	2	0	0	0	0	0	0
Dogs (a)	87	29	10	34.5	0	0	0	0	0	0
	88	23	0	0.0	0	0	0	0	0	0
Donkeys	87	13	3	23.1	0	0	0	0	0	0
Goats	87	75	0	0	0	0	2	2.7	0	0
Sheeps	87	17	0	0	1	5.9	1	5.9	0	0
Rodents	87	379	17	4.5	0	0.0	0	0.0	2	0.5
	88	115	2	1.7	0	0.0	0	0.0	2	1.7
Mastomys	87	265	12	4.5	0	0.0	0	0.0	2	0.8
	88	67	1	1.4	0	0.0	0	0.0	0	0.0
Arvicanthis	87	98	9	9.2	0	0.0	0	0.0	0	0.0
	88	36	3	8.3	0	0.0	0	0.0	0	0.0
Shrew	87	5	0	0.0	0	0.0	0	0.0	0	0.0
	88	1	0	0.0	0	0.0	0	0.0	0	0.0
Taterillus	87	4	0	0.0	0	0.0	0	0.0	0	0.0
	88	6	0	0.0	0	0.0	0	0.0	0	0.0
Praomys	87	3	0	0.0	0	0.0	0	0.0	0	0.0
	88	4	0	0.0	0	0.0	0	0.0	0	0.0
Other (b)	87	4	0	0.0	0	0.0	0	0.0	0	0.0
	88	1	1	0.0	0	0.0	0	0.0	0	0.0

a) Village prevalence (1987): 2/7 (28.5%) Gordil, 8/22(36.4%)Ouandjia. 0/5 Gordil (0%) and 0/18 Ouandjia (0%).

b)(1987) Myomys, Gerbil, Lemniscomys, Grapiosus; (1988) Aetomys.

D. DISCUSSION

Collectively, our serological data indicate that the filoviruses (Ebola virus, Marburg virus, or a serologically related member of the group) are active in the Central African Republic. Significant antibody prevalences are consistently observed in select populations and exposure seems to occur early in life. The prevalence of antibodies in children less than 6 years old, and the fact that several families have numerous positive members whereas some families are completely seronegative are consistent with possible occurence of inter-human contamination.

The circulation of a member of the filovirus group seems not to be associated with notable hemorrhagic disease.

In year 1988, we could interrogate 109 persons in North Vakaga district, and among them, 3 persons who seroconverted between 1986 and 1987, they all denied having suffered of a severe illness during this period, and in particular never experimented hemorrhage signs (the 3 persons were found seronegative in 1988).

In the Ombella Mpoko district (surrounding Bangui), during the same period we examined and bled 214 febrile people : none claimed to have any hemorrhagic signs.

We must admit that infection by a member of filovirus group can be associated with no illness or with a mild febrile syndrom, indifferenciable from malaria or "dengue-like" syndrom. Except for one fatal case observed in Gordil in 1985, no severe febrile illness compatible with infection by a virus responsible for hemorrhagic disease has been reported by the inhabitants of Ouandjia and Gordil.

These observations don't exclude the possibility of a Filovirus activity and especially Ebola or Marburg virus since neither the severity nor the clinical spectrum of natural occuring infections have been definitively described. A continuous clinical, epidemiological, virological surveillance remains necessary.

Animal hosts for the filoviruses have been systematically sought. On the basis of species in which antibodies have been detected, cattle, chicken, dogs, donkeys, rodents can be infected in natura by a filovirus. However their possible role in the virus transmission to man is not easy to assess on the sole basis of serological results obtained by IFA test. As for human no filovirus isolation has been obtained from animals.

As regards to cattle, the low prevalence in a group of M'bororo people, living in contact with their livestock herd and who were present in Vakaga for at least 3 years, is not in favour of an active role in the virus transmission. This observation must be confirmed.

Rodents were especially studied because their important role in the transmission of numerous viruses. A low prevalence was observed in 1987 among rodents caught in the village and living in contact with man. In 1988 rodents were caught in croplands owning to families with numerous seropositive and in croplands owning to families with no seropositive: no difference has been found as regards to the seropositivity rate in both rodents populations, suggesting that rodents may don't play an essential role in the transmission of filovirus to man. As our capacities did not permit to study several important factors such as seasonal vegetation, annual rainfall, predator frequency, rodents densities ..., this observation must also be confirmed by longer field missions and assistance of a mammalogist.

Results are not so clear concerning dogs : a high prevalence rate obtained in 1987 (36.4% in Ouandja) led us to suggest the possibility of a virus cycle including dogs. Since this high positivity rate was not confirmed in 1988, further studies are needed to evaluate the role of dogs in the virus cycle.

E. RECOMMENDATIONS

The goal of the collaboration between the Institut Pasteur in Bangui and USAMRIID was to define the epidemiology, ecology and pathogenicity of the filoviruses.

The main following results have been obtained :

- an agent antigenically related to the Filovirus group is active in the Vakaga area in Central African Republic (as well as in *other regions*), as demonstrated by high seroprevalence rates in human population;

- as very few hemorrhagic diseases are noticed to the Institute Pasteur despite a continuous surveillance program, an infection by this agent doesn't seem usually the cause of notable hemorrhagic disease. On the basis of numerous interviews of seropositives, an infection by this virus seems usually not associated with a severe illness;

- despite numerous isolation attempts, especially from sera samples collected on patients presenting at least an acute febrile syndrome compatible with a virus infection, no filovirus strain has been isolated. One cannot say if the agent responsible for positive serologies is Ebola, Marburg or an unknown antigenically related strain;

- infection occurs early in the life, as demonstrated by antibody prevalence in children less than 6 years old. Differences according to sex are still unclear;

- in the villages studied antibody prevalence seems to be associated with lower population densities, i.e. outlying areas versus centre of villages;

- the seropositivity rate is not equally distributed through the population. Some families have a high seropositivity rate (up to 50% or more), others have no seropositive member;

- concerning a possible animal reservoir, rodents, cattle, chicken, dogs can be infected.

Further studies are needed :

1. the specificity of filovirus serologic reaction must be studied, in order to know whose viral proteins are recognized by antibodies detected by immunofulorescence assay. It would be especially important to determine whether these antibodies are specific of Ebola or Marburg virus, and if not, whether they can neutralize an infection by these viruses. This work is only possible in a specialized laboratory;

2. obviously efforts should be made to isolate the viral agent detected by the filovirus serology. Isolating a weakly or non-pathogenic agent without a known animal reservoir will be probably difficult. Our current approach is to attempt virus isolations from suggestive clinical specimens obtained from different places in and out the Central African Republic;

3. continuous surveillance of human and animal populations is also necessary to establish risk factors and to understand the epidemiology of filovirus. This study would consist in spending a long time (1 or 2 months), with the assistance of an anthropologist and a mammalogist in order to make an inventory of possible contamination sources (animal and others), study the socio-cultural factors of transmission, evaluate the possibility of inter-human contamination.

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